CDNA CHIP FOR SCREENING SPECIFIC GENES AND ANALYZING THEIR FUNCTION IN SWINE

Technical Field

The present invention relates to a cDNA chip for screening and function analysis of swine genes. More particularly, the present invention relates to a technique for screening swine genes and analyzing their functions by preparing a cDNA chip comprising a probe to detect marker genes specifically expressed in the muscle and fat tissues of swine, in which the probe comprises 4434 ESTs isolated from the tissues and is capable of complementarily binding to the marker genes, and application of the technique for swine improvement.

Background Art

For creation of the value added in the hog raising farm, acquisition of foreign currencies, and raise of competitiveness of the domestic hog raising industry which depends on foreign countries for feed and swine supplies, it is a necessary assignment to obtain a swine breed with excellent quality. To solve this assignment, the present inventors have screened meat quality-related specific genes in swine and made a cDNA chip using the same. In the production of transformed swines using such specific genes, and branding and popularization of the new breeds, thereby creating highly added value of the hog-raising farm, the function analysis of swine genes is an indispensable step.

For the last several years, the studies of linkage map and physical maps of the pig genome have been remarkably advanced. The PiGMaP Project was initiated in Europe and now involves 18 European labs and a total of 7 other labs from the U.S., Japan and Australia. At present, nearly 1,800 markers and genes have been mapped in pigs (Archibald et al. 1994; Marklund et al. 1996; Rohrer et al. 1996). The physical genetic map in the pig currently consists of over 600 genes. Several quantitative trait loci (QTL) scans and locations of candidate genes were found on chromosome and major genes associated with traits of economic traits in the pig were identified. The genes related to growth and back fat exist on chromosomes 3, 4, 5, 6, 7, 8, 13 and 14, the genes related to meat quality exist on chromosomes 2, 3, 4, 6, 7, 12 and 15, and the genes related to reproduction traits exist on chromosomes 4, 6, 7 and 8. In addition, ESR and PRLR, candidate genes related to litter size, FUT1, a gene for disease resistant, SLA, NRAMP, and KIT, a gene for coat color, and MSHR were identified.

Concretely looking in to the main traits of the pig, the important the growth related genes were analyzed using a Wild Boar and Large White three generation family and the analysis revealed major QTL accounting for 20% of the phenotypic variance for back fat and abdominal fat on chromosome 4. A QTL for growth was found on chromosome 13 accounting for 7% to 12% of the phenotypic variation. By candidate gene analysis, PRT1 was found to be associated with back fat and birth weight and it maps in the center of chromosome 13 by Andersson et al. The pig MHC is positioned on chromosome 7. Associations

between MHC haplotypes and several traits have been reported over the years. These have been confirmed, in part, using MHC class DNA probes. Recently, QTL related to growth and back fat traits was found on chromosome 7 in Chinese crosses. The QTL for back fat and birth weight are shown to be positioned near the region of TNFA and S0102. The overall results to date suggest that at least one growth and back fat QTL exists in this region. Other results have included a growth trait QTL on chromosome 6, but it seems to be associated with the effect caused by the RYR1 gene causing malignant hyperthermia or other unknown genes around RYR1. Some similar associations have been reported for chromosomes 3, 6, 8 and 14. Additionally, according to Gerbens and Tepas, it was reported that the fatty acid absorbing protein in the heart and major genetic factors are associated with average daily gain. Other candidate genes, including Leptin CCK and CCKAR have been mapped and may prove to be associated with appetite, fatness and growth traits.

Next, in the connection with the meat quality traits, it has been known that PSE pork is caused by RYR1 on chromosome 6. This has been demonstrated to be associated with several meat quality traits related to PSE in an F2 population originating from a Pietrain background. Focus has also centered on Hampshires for the RN gene which is associated with increased glycogen content and lower pH in the meat. The RN gene has now been mapped to chromosome 15 and is located between flanking markers. Andersson and colleagues have conducted one of the most complete QTL scans for meat quality using 234 markers on 191 F2 animals for mapping. QTL for several meat

quality traits (pH, water holding capacity and pigmentation) were found to be on chromosomes 2 and 12. Rothschild and colleagues report that meat color and firmness scores are associated with regions on chromosomes 4 and 7. Additional associations with meat quality traits have been reported on chromosome 7 and for number of muscle fibers on chromosome 3. The activity of Malic enzyme, a lipogenic enzyme in muscle has been shown to be associated with the SLA complex on chromosome 7. Furthermore, a major QTL for androstenone level which is associated with boar taint in the region of the SLA complex was found.

Among candidate genes investigated for muscle quality is the HABP gene which may be associated with intramuscular fat. Many genes were found for myogenin.

Next, for reproduction traits, since larger resource families and time are required to obtain information thereon, which make the study difficult, results of QTL scan for these traits are limited. Wilkie et al. reported QTL for uterine length and ovulation rate, though in different chromosomal positions. Rathje et al. reported a QTL related to ovulation rate on chromosome 8, but there were some differences from the ovulation-related QTL observed by Wilkie and colleagues. In the French QTL experiment by Milan et al., a QTL for increased litter size of one piglet was found in the same location on chromosome 8 as Rathje. The large ovulation rate-related QTL on chromosome 8 is of interest as it mapped to the same region to the Booroola gene in sheep. Interestingly, Short et al. also found significant effects of this locus for litter size in commercial lines. Limited chromosome QTL analyses for

reproductive QTL have been conducted on chromosomes 4, 6, 7, 13 and 15. It has been clearly demonstrated that the estrogen gene is significantly associated with litter size. Though genetic effects vary according to the breeds, the increase is 1.15 pig/litter in Meishan synthetics and 0.42 pigs/litter in Large White lines. More recent results have demonstrated that the prolactin receptor locus is significantly associated with litter size.

Finally, for disease resistance and immune response traits, to date, QTL scans for disease resistance or immune response QTL have been limited. Some immunity related QTL have been identified. Also, a QTL for cortisol level which may be related to stress and perhaps immune response, has been mapped to the end of chromosome 7. Two alpha genes FUT1 and FUT2 on porcine chromosome 6 have been identified. Vögeli and colleagues have disclosed a marker showing a polymorphism which is closely linked to ECF18R gene in Large White, Landrace, Hampshire, Duroc and Pietrain pigs and it could be a good marker for marker assisted selection of *E. coli* F18 adhesion resistant animals in these breeds. Recently, it has been reported that the SLA complex on chromosome 7 is associated with resistance to infections with *Trichinella spiralis* but not resistance to toxoplasmosis. The NRAMP1 gene, known to be associated with resistance to *Salmonella* challenge in mice, has been recently mapped to pig chromosome 15. Genes associated with human disorders, which have been identified in the pig, include clotting factor IX and the hypercholesterolaemia gene.

Considering the foregoing, the present inventors made efforts to find candidate genes for genetic improvement of economic traits in swine, that is, for development of swine with excellent growth performance, meat quality, disease resistance and reproductive performance.

Up to now, several technologies to analyze gene expression at the mRNA level such as northern blotting, differential display, sequential analysis of gene expression and dot blot analysis have been used to examine the genetic difference in swine. However, these methods have disadvantages which are not suitable for simultaneous analysis of a plurality of expressed products. In recent, a new technology such as cDNA microarray to overcome such disadvantages has been developed. The cDNA microarray becomes one of the strongest means to study gene expression in various living bodies. This technology is applied to simultaneous expression of numerous genes and discovery of genes in a large scale, as well as genetic polymorphism screening and mapping of genetic DNA clone. It is a highly advanced RNA expression analysis technology to quantitatively analyze RNA transcribed from already known or not-known genes. Such microarray uses a DNA chip. The gene chip is classified into cDNA (200-500 bp) chips and oligonucleotide (15-100 bp) chips according to the nucleotide to be detected. Also, according to the preparation method, it is classified into robot printing chips such as pin microarray or ink-jet and photolithography chips using the semiconductor production process. The cDNA chip peculiarly distinguishes a gene having a complementary sequence by

attaching a full-length sequence of ORF (Open Reading Frames) or EST (Expression Sequence Tags) to a slide.

Disclosure of Invention

Therefore, an object of the present invention is to provide a cDNA chip comprising a probe fixed thereon to detect marker genes specifically expressed in the muscle and fat tissues of swine, thereby being applied to swine applied improvement as well as screening and function analysis of swine genes, in which the probe is capable of complementarily binding to the marker genes.

It is another object of the present invention to provide expression profiles of marker genes which are related to economic traits of swine.

It is a further object of the present invention to provide means for comparison of gene expression according to breeds and tissues in swine, genetic mutation screening, genetic polymorphism interpretation, development of a new drug for treatment of diseases and disease diagnosis using the cDNA chip according to the present invention.

According to the present invention, the above-described objects are accomplished by, to prepare a probe DNA, preparing a probe DNA comprising extracting RNA from the muscle and fat tissues of swine and prepare cDNA therefrom, cloning 4434 ESTs and analyzing and screening the nucleotide sequence in the data base, amplifying the ESTs by PCR, followed isolation and purification, and immobilizing (spotting) the product with 300 yeast control genes on a slide using a DNA chip array to prepare a DNA chip, hybridizing a target DNA prepared by

binding a fluorescent material to total RNA isolated from the muscle and fat tissues of swine with the prepared probe DNA, followed by scanning and analysis of image files, and examining profiles of genes specifically expressed in the muscle and fat tissues.

The present invention comprises the steps of preparation of ESTs from the muscle and fat tissues of swine and identification of sequence information; amplification of ESTs by PCR, followed by isolation and purification; preparation of a DNA chip by immobilizing the ESTs on a slide using a DNA chip array; hybridization of a fluorescent-labeled target DNA (ESTs) from total RNA isolated from the muscle and fat tissues of swine with the probe DNA, followed by scanning and image file analysis; and examination of the expression profiles of genes specifically expressed in the muscle and fat tissues of swine.

The cDNA chip for screening and function analysis of swine genes is prepared by the following steps: preparing cDNA from total RNA isolated from the muscle and fat tissues of swine; cloning 4434 ESTs thereof and analyzing and screening the obtained sequences in the database; amplifying the ESTs by PCR, followed by isolation and purification; and spotting the 4434 ESTs on a slide using a DNA chip array to prepare a DNA chip.

The cDNA chip for screening and function analysis of swine genes according to the present invention comprises a probe capable of complementarily binding to cDNA or RNA of marker genes and a substrate on which the probe is immobilized.

According to the present invention, the probe DNA immobilized on a DNA microarray of the cDNA chip for screening and function analysis of swine comprises 4434 ESTs isolated from the muscle and fat tissues of swine.

The substrate is preferably a polymer film such as silicone wafer, glass, polycarbonate, membrane, polystyrene or polyurethane. The DNA microarray according to the present invention may be prepared by immobilizing a probe on a substrate by a conventional method for preparing a DNA microarray, including photolithography, piezoelectric printing, micro pipetting, spotting and the like. In the present invention, the spotting method is used.

The marker genes to be detected from the probe DNA immobilized on the DNA microarray of the cDNA chip for screening and function analysis of swine genes according to the present invention include 1-alpha dynein heavy chain, 19 kDa-interacting protein 3-like, actin, actin alpha 1, actin gamma 2, annexin A2, annexin V, annexin Ⅱ beta-myosin heavy chain mRNA, calpain large polypeptide L2, collagen, collagen alpha 1, collagen alpha 2, collagen alpha V, discs large (*Drosophila*) homologue 5, fibronectin, heparan sulfate proteoglycan 2, lamin A/C, myosin, myosin heavy chain, myotubularin related protein 4, procollagen-proline, acidic secreted protein, tropomyosin, tropomyosin alpha chain, troponin C, tubulin beta chain and vimentin, which are related to the cellular structure and motility.

The marker genes to be detected from the probe DNA immobilized on the DNA microarray of the cDNA chip for screening and function analysis of swine genes according to

the present invention include aldolase A, carbonate dehydratase, cytochrome C, cytochrome C oxidase subunit I, cytochrome-C oxidase, fructose-1, 6-bisphosphatase, L-lactate dehydrogenase M chain, LIM domains 1 protein, NADH dehydrogenase, NADH-ubiquinone oxidoreductase chain 1, NADH4L, octanoyltransferase (COT), phosphoarginine phosphatase, phosphoglucomutase isoform 2 mRNA, protein-tyrosine kinase, pyruvate kinase, sarcolipin, tyrosine phosphatase type IVA, UDP glucose pyrophosphorylase, glycogen phosphorylase b and superoxide dismutase, which are related to the metabolism.

The marker genes to be detected from the probe DNA immobilized on the DNA microarray of the cDNA chip for screening and function analysis of swine genes according to the present invention include elongation factor 1 alpha, elongation factor 1 alpha 1, enolase 3, repetitive DNA sequence element RPE-1, reticulum protein, ribonucleoprotein polypeptide B, ribosomal protein, ribosomal protein L18a, ribosomal protein P0, transfer RNA-Trp synthetase, translation initiation factor eif1, LIM domains 1 protein and tissue inhibitor of metalloproteinase 3, which are related to the expression of genes and proteins.

The marker genes to be detected from the probe DNA immobilized on the DNA microarray of the cDNA chip for screening and function analysis of swine genes according to the present invention include complete mitochondrial DNA, mitochondrion, potassium channel and similar to creatine kinase, which are related to the signaling and communication of cells.

The marker genes to be detected from the probe DNA immobilized on the DNA microarray of the cDNA chip for screening and function analysis of swine genes according to the present invention include protease and cystein 1, which are related to the cell division.

The marker genes to be detected from the probe DNA immobilized on the DNA microarray of the cDNA chip for screening and function analysis of swine genes according to the present invention include Interleukin-2 receptor alpha chain, Kel-like protein 23 and MHC class I SLA genomic region, which are related to the immune response.

The marker genes to be detected from the probe DNA immobilized on the DNA microarray of the cDNA chip for screening and function analysis of swine genes according to the present invention include the nucleotide sequences of growth factors I, II, III, IV and V as set forth in SEQ ID NOs: 1 to 5, which are related to growth.

Also, the present invention provides a kit for screening and function analysis of swine genes comprising the cDNA chip, Cy5-dCTP or Cy3-dCTP bound cDNA from RNA of the tissue to be screened, a fluorescence scanning system and computer analysis system.

By the method for detecting the expression profiles of specific genes using the cDNA chip for screening and function analysis of swine genes according to the present invention, it is possible to evaluate meat quality of swine by analyzing marker genes expressed in a certain cell. Also, the method can be used for development of swine with improved growth performance by using the detected growth-specific genes of

swine, and for disease diagnosis of swine and development of drug by identifying the profiles of genes involved in the general mechanism and the immune response to disease resistance of cells.

Best Mode for Carrying Out the Invention

Now, the construction of the present invention will be explained through the following Examples in detail. However, the present invention is not limited thereto.

[Example]

Example 1: Screening of swine genes and construction of cDNA chip for function analysis

In order to prepare a cDNA chip for screening and function analysis of swine genes, a probe DNA was prepared by subjecting total RNA isolated form the muscle and fat tissues of Kagoshima Berkshire to PCR to obtain 4434 ESTs, cloning the ESTs, analyzing and screening their sequences in the database, amplifying the ESTs by PCR, followed by isolation and purification, and immobilizing the product on a slide using a DNA chip array to obtain a cDNA chip for screening and function analysis of swine genes.

Preparation Example 1: Preparation and array of probe DNA

Firstly, a probe DNA, which was cDNA amplified by PCR, was prepared and attached to a slide glass. Total RNA was extracted from the muscle and fat tissues of the longissimus dorsi of *Kagoshima Berkshire* (body weight of 30 kg and 90 kg) using a RNA extraction kit (Qiagen, Germany) according to the

manual and mRNA was extracted using an oligo (dT) column. The extracted mRNA sample was subjected to RT-PCR using SP6, T3 forward primer, T7 reverse primer (Amersham Pharmacia Biotech, England) to synthesize cDNA. The total volume of each PCR reactant was 100 $\mu\ell$. 100 pM of a forward primer and a reverse primer were each transferred to a 96-well PCR plate (Genetics, England). Each well contained 2.5 mM dNTP, 10×PCR buffer, 25 mM $MgCl_2$, 0.2 μg of DNA template, 2.5 units of Taq polymerase. PCR was performed in GeneAmp PCR system 5700 (AB Applied BioSystem, Canada) under the following conditions: total 30 cycles of 30 seconds at 94℃, 45 seconds at 58℃, and 1 minute at 72℃.

The size of the amplified DNA was identified by agarose gel electrophoresis. The PCR product was precipitated with ethanol in 96-well plate, dried and stored at -20℃

Total 4434 cDNAs (ESTs), prepared as described above, were cloned to analyze nucleotide sequences of genes which swine has and their genetic information was identified from the database at NCBI. The genes having information were isolated and purified by PCR. The genetic locus and map for the total 4434 cDNAs (ESTs) were constructed. The total 4434 cDNAs (ESTs) and 300 yeast control genes were arrayed in an area of 1.7 cm^2. Then, the probe DNA was spotted on a slide glass for microscope (produced by Corning), coated with CMT-GAPSTM aminosilane using Microgrid II (Biorobotics). The probe DNA was printed onto Microgrid II using a split pin. The pin apparatus was approached to the well in the microplate to inject the solution into the slide glass (1 to 2 nL). After printing of the probe DNA, the slide was dried and the

spotted DNA and the slide were UV cross-linked at 90 mJ using Stratalinker TM (Stratagene, USA), washed twice with 0.2% SDS at room temperature for 2 minutes and washed once with third distilled water at room temperature for 2 minutes. After washing, the slide was dipped in a water tank at 95℃ for 2 minutes and was blocked for 15 minutes by adding a blocking solution (a mixture of 1.0 g of $NaBH_4$ dissolved in 300 mL of phosphate buffer (pH7.4) and 100 mL of anhydrous ethanol). Then, the slide was washed three times with 0.2% SDS at room temperature for 1 minute and once with third distilled water at room temperature for 2 minutes and dried in the air.

The marker genes which can be detected from the probe DNA prepared from the muscle and fat tissues of swine are as follows:

1) Genes for the cellular structure and motility

1-alpha dynein heavy chain, 19 kDa-interacting protein 3-like, actin, actin alpha 1, actin gamma 2, annexin A2, annexin V, annexin II, beta-myosin heavy chain mRNA, calpain large polypeptide L2, collagen, collagen alpha 1, collagen alpha 2, collagen alpha V, discs large (*Drosophila*) homologue 5, fibronectin, heparan sulfate proteoglycan 2, lamin A/C, myosin, myosin heavy chain, myotubularin related protein 4, procollagen-proline, acidic secreted protein, tropomyosin, tropomyosin alpha chain, troponin C, tubulin beta chain and vimentin

2) Genes for the metabolism

Aldolase A, carbonate dehydratase, cytochrome C, cytochrome C oxidase subunit I, cytochrome-C oxidase,

fructose-1,6-bisphosphatase, L-lactate dehydrogenase M chain, LIM domains 1 protein, NADH dehydrogenase, NADH-ubiquinone oxidoreductase chain 1, NADH4L, octanoyltransferase (COT), phosphoarginine phosphatase, phosphoglucomutase isoform 2 mRNA, protein-tyrosine kinase, pyruvate kinase, sarcolipin, tyrosine phosphatase type IVA, UDP glucose pyrophosphorylase, glycogen phosphorylase b and superoxide dismutase

3) Genes for the expression of genes and proteins

Elongation factor 1 alpha, elongation factor 1 alpha 1, enolase 3, repetitive DNA sequence element RPE-1, reticulum protein, ribonucleoprotein polypeptide B, ribosomal protein, ribosomal protein L18a, ribosomal protein P0, transfer RNA-Trp synthetase, translation initiation factor eif1, LIM domains 1 protein and tissue inhibitor of metalloproteinase 3

4) Genes for the signaling and communication cells

Complete mitochondrial DNA, mitochondrion, potassium channel and similar to creatine kinase

5) Genes for the cell division

Protease and cystein 1

6) Genes for the immune response

Interleukin-2 receptor alpha chain, Kel-like protein 23 and MHC class I SLA genomic region

7) Genes for growth

Growth factor I, II, III, IV and V as set forth in SEQ ID NO: 1 to 5

8) Others

cDNA flj13323 fis, KIAA0182 protein, KIAA1096 protein,AC015998, AR078G01iTHYEG01S, Cn26h08.x1, COI, DJ466P17.1.1(Laforin), foocen-m, HWM012cA.1, hypothetical protein, mandarina library, MARC 1PI, MARC 2PIG, MR1-AN0039-290800-004-a01, NIH_MGC_4, NIH_MGC_65, NIH_MGC_77, NIH_MGC_77, Peripheral blood cell cDNA library, putative, reinhardtii CC-1690, small intestine cDNA library, thymosin beta-4 mRNA, unknown, unnamed protein product, chromosome 14 DNA sequence, integrin beta-1 subunit, reinhardtii CC-1690.

Experimental Example 1: Expression profile screening of tissue specific genes using the cDNA chip according to the present invention

The expression profiles of genes specifically expressed in the muscle and fat tissues of swine were examined using the cDNA chip prepared in Example 1. The muscle tissue on the longissimus dorsi area was taken from the *Kagoshima Berkshires* having body weights of 30 kg and 90 kg. The fat tissue was taken from the Kagoshima Berkshire having a body weight of 30 kg. The muscle and fat tissues were cut into 5~8 mm length, frozen with liquid nitrogen and stored at -70℃.

Total RNA was isolated from 0.2 to 1.0 g of the experimental group and the control group according to the manual of Trizol ™ kit (Life Technologies, Inc.) to prepare the target DNA. Trizol™ was added to the tissue in an amount

of 1 mL of Trizol™ per 50 to 100 mg of tissue and disrupted using a glass-Teflon or Polytron homogenizer. The disrupted granules were centrifuged at 4℃ at a speed of 12,000 g for 10 minutes and 1 mL of the supernatant was aliquoted. 200 μℓ of chloroform was added to each aliquot, voltexed for 15 seconds, placed on ice for 15 minutes and centrifuged at 4℃ at a speed of 12,000 g for 10 minutes. Chloroform of the same amount was again added thereto, voltexed for 15 seconds, placed on ice for 15 minutes and centrifuged at 4℃ at a speed of 12,000 g for 10 minutes. The supernatant was transferred to a new tube. 500 μℓ of isopropanol was added to the tube, voltexed and placed on ice for 15 minutes. The ice was cooled and centrifuged at 4℃ at a speed of 12,000 g for 5 minutes. The supernatant was removed, mixed with 1 mL of 75% cold ethanol and centrifuged at 4℃ at a speed of 12,000 g for 5 minutes. The supernatant was removed, freeze-dried on a clean bench for 30 minutes and take into 20 μℓ of RNase-free water or DEPC water to dissolve RNA. The total DNA concentration was set to 40 μg/17 μℓ for electrophoresis.

The target DNA was prepared according to the standard first-strand cDNA synthesis. Briefly, according to the method described by Schuler (1996), 40 μg of total RNA and oligo dT-18mer primer (Invitrogen Life Technologies) were mixed, heated at 65℃ for 10 minutes and cooled at 4℃ for 5 minutes. Then, 1 μℓ of a mixture of 25 mM dATP, dGTP and dTTP, 1 μℓ of 1 mM dCTP (Promega) and 2 μℓ of 1 mM cyanine 3-dCTP or 2 μℓ of 1 mM cyanine 5-dCTP, 20 units of RNase inhibitor (Invitrogen Life Technology), 100 units of M-MLV RTase, 2 μℓ of 10 × first strand buffer were added thereto and mixed with a pipette.

The reaction mixture was incubated at 38℃ for 2 hours and the non-bound nucleotide was removed by ethanol precipitation. Here, DEPC treated sterile water was used.

The slide, prepared above, was pre-hybridized with a hybridization solution (5×SSC, 0.2% SDS, 1 mg/mL herring sperm DNA) at 65℃ for 1 hour. The target DNA labeled with cyanine 3 (Cy-3) and cyanine 5 (Cy-5) was re-suspended in 20 ㎕ of the hybridization solution and denatured at 95℃ for 2 minutes. Then, the slide was hybridized with the solution at 65℃ overnight. The hybridization was performed in a humidity chamber covered with a cover glass (Grace Bio-Lab).

After hybridization, the slide was washed 4 times with 2×SSC, 0.1% SDS at room temperature for 5 minutes while vigorously stirred in a dancing shaker. Then the slide was washed twice with 0.2×SSC for 5 minutes and with 0.1×SSC for 5 minutes at room temperature.

The slid was scanned on ScanArray 5000 (GSI Lumonics Version 3.1) with a pixel size of 50 ㎛. The target DNA labeled by cyanine 3-dCTP was scanned at 565 nm and the target DNA labeled by cyanine 5-dCTP was scanned at 670 nm. Two fluorescence intensities were standardized by linear scanning of the cyanine 3-dCTP- and cyanine 5-dCTP-labeled spots. The slide was again scanned on Scanarray 4000XL with a pixel size of 10 ㎛. The resulting TIFF image files were analyzed on Quantarray software version 2.1 and the background was automatically subtracted. The intensity of each spot was converted into Microsoft Excel on Quantarray.

The entire expression pattern of ESM (early stage muscle) genes expressed in the early stage in the muscle and

fat tissues of swine were compared with those of ASM (adult stage muscle) gene expressed in the adult stage and ESF (early stage fat) gene expressed in the early stage. The "ESM-specific" and "ASM-specific" genes are shown in Table 1 and the "ESF-specific" genes are shown in Table 2. 20 genes showed a 5 times higher expression level in ASM, as compared to ESM. Also, 18 genes showed a 5 to 10 times higher expression level in ESF, as compared to ESM, and a 5 to 10 times higher expression level in ESM, as compared to ASM.

Also, the 5 following growth specific genes specifically expressed in the muscle and fat tissues of swine were found.

1. GF (growth factor) I gene: SEQ ID NO 1

gagaccagca aatactatgt gaccatcatt gatgccccag gacacagaga cttcatcaaa 60

aacatgatta caggcacatc ccaggctgac tgtgctgtcc tgattgttgc tgctggtgtt 120

ggtgaatttg aagctggtat ctccaagaac gggcagaccc gcgagcatgc tcttctggct 180

tacaccctgg gtgtgaaaca gctgattgtt ggtgtcaaca aaatggattc caccgagcca 240

ccatacagtc agaagagata cgaggaaatc gttaaggaag tcagcaccta cattaagaaa 300

attggctaca accctgacac agtagcattt gtgccaattt ctggttggaa tggtgacaac 360

atgctggagc caagtgctaa tatgccttgg ttcaagggat ggaaagtcac ccgcaaagat 420

ggcagtgcca gtggcaccac gctgctggaa gctttggatt gtatcctacc accaactcgt 480

ccaactgaca agcctctgcg actgcccctc caggatgtct ataaaattgg aggcattggc 540

actgtccctg tgggccgagt ggagactggt gttctcaaac ctggcatggt ggttaccttt 600

gctccagtca atgtaacaac tgaagtcaag tctgttgaaa tgcaccatga agctttgagt

2. GF (growth factor) II gene: SEQ ID NO 2

gctgactgat cgggagaatc agtctatctt aatcaccgga gaatccgggg caggaaagac 60

tgtgaacacg aagcgtgtca tccagtactt tgccacaatc gccgtcactg gggagaagaa 120

gaaggaggaa cctactcctg gcaaaatgca ggggactctg gaagatcaga tcatcagtgc 180

caacccccctg ctcgaggcct ttggcaacgc caagaccgtg aggaacgaca actcctctcg 240

ctttggtaaa ttcatcagga tccacttcgg taccactggg aagctggctt ctgctgacat 300

cgaaacatat cttctagaga agtctagagt cactttccag ctaaaggcag aaagaagcta 360

ccacattttt tatcagatca tgtctaacaa gaagccagag ctcattgaaa tgctcctgat 420

caccaccaac ccatatgact acgccttcgt cagtcaaggg gagatcactg tccccagcat 480

tgatgaccaa gaggagctga tggccacaga tagtgccatt gaaatcctgg

3. GF (growth factor) Ⅲ gene: SEQ ID NO 3

gttgttcctt taaatatgat gttgccacaa gctgcattgg agactcattg cagtaatatt 60

tccaatgtgc cacctacaag agagatactt caagtctttc ttactgatgt acacatgaag 120

gaagtaattc agcagttcat tgatgtcctg agtgtagcag tcaagaaacg tgtcttgtgt 180

ttacctaggg atgaaaacct gacagcaaat gaagttttga aaacgtgtga taggaaagca 240

aatgttgcaa tcctgttttc tgggggcatt gattccatgg ttattgcaac ccttgctgac 300

cgtcatattc ctttagatga accaattgat cttcttaatg tagctttcat agctgaagaa 360

aagaccatgc caactacctt taacagagaa gggaataaac agaaaaataa atgtgaaata 420

ccttcagaag aattctctaa agatgttgct gctgctgctg ctgacagtcc taataaacat 480

tcagtgtacc agatcgaatc acaggaaggg cgggactaaa ggaactacaa gctgttagc

4. GF (growth factor) Ⅳ gene: SEQ ID NO 4

catttatgag ggctacgcgc tgccgcacgc catcatgcgc ctggacctgg cgggccgcga 60

tctcaccgac tacctgatga agatcctcac tgagcgtggc tactccttct gaccacagct 120

gagcgcgaga tcgtgcgcga catcaaggag aagctgtgct acgtggccct ggacttcgag 180

aacgagatgg cgacggccgc ctcctcctcc tccctggaaa agagctacga gctgccagac 240

gggcaggtca tcaccatcgg caacgagcgc ttccgctgcc cggagacgct cttccagccc 300

tccttcatcg gtatggagtc ggcgggcatt cacgagacca cctacaacag catcatgaag 360

tgtgacatcg acatcaggaa ggacctgtat gccaacaacg tcatgtcggg gggcaccac

5. GF (growth factor) Ⅴ gene: SEQ ID NO 5

tatatagaac cgaatcacgt acactgggcc tgaccaagca gggccaaaac aaggcaacct 60

aggaggttat aaaataggta tacgcgcgct gacacataca tactcactac ccgaacgcgg 120

ggacaactag ggctccgcca taagccatcc tttcctggtc gtcgatgttg cgggctgcag 180

ttatagggct gccaaccgcc atacacacct taccagccac ttattaagtt acatccacga 240

gggctctgta ccacccctaa gcagtggcag tggtagccgc tgcccgctta ccctgcgcag 300

tgttggtgct agctccgtcc taagcttccc cgatagccgc cgctttttac acaccatcgg 360

cggactagac accgttggtt gcagcgtaag cgtctatggt agcagctgcg gcgaccgccg 420

tgtagccagc ttactacatg ttagtttcag caaccaccct gccaataccc gtgttcccta 480

ctccaactct gtcggtttca gccgcag

【Table 1】

Expression ratio of differentially expressed genes between ESM and ASM

ESTs No.	Accession No.†	Description**	Ratio of gene expression ESM(30) / ASM(90)
Cellular structure and motility			
SM2149	CAB56598	1-alpha dynein heavy chain	-2.1
SM781	NP_033891	19 kDa-interacting protein 3-like	+2.1
SM635	BAB19361	Actin	+3.4
SM713	AAA51586	Actin	+6.3
SM106	P53506	Actin	+8.8
SM1068	AAF20165	Actin	+5.3
SM363	B25819	Actin	+4.3
SM768	X52815	Actin	+3.4
SMk77	NM_001100	Actin, alpha 1	+15.1
SM128	NP_033740	Actin, gamma 2	+6.9
SM902	BC001748	Annexin A2	-3.2
SM846	P81287	Annexin V	-2.8
SM653	P04272	Annexin II	-2.2
SMk340	U75316	Beta-myosin heavy chain mRNA	+3.0
SM1605	AAF99682	Calpain large polypeptide L2	+4.7
SM541	NP_000079	Collagen	-3.2
SM715	L47641	Collagen	-6.8
SM430	Q9XSJ7	Collagen alpha 1	-6.8
SM758	CGHU1S	Collagen alpha 1	-2.1
SM62	CGHU2V	Collagen alpha 2	-3.2
SM949	O46392	Collagen alpha 2	-3.3
SM410	CAA28454	Collagen (alpha V)	-2.3
SM1651	XM_039583	Discs, large (Drosophila) homolog 5	-2.0
SM1050	AAA30521	Fibronectin	-2.4

SM491	NM_005529	Heparan sulfate proteoglycan 2	-2.2
SM1573	XM_044160	Lamin A/C	+2.6
SMk55	NP_006462	Myosin	+3.9
SMk338	P79293	Myosin heavy chain	+2.0
SMk168	AB025261	Myosin heavy chain	+9.0
SM1732	NP_004678	Myotubularin related protein 4	+3.8
SM1691	NP_000908	Procollagen-proline	-2.3
SM690	NP_003109	Secreted protein, acidic	-4.4
SMk173	X66274	Tropomyosin	+2.6
SM141	CAA38179	Tropomyosin	+2.7
SMk51	P18342	Tropomyosin alpha chain	+9.6
SM1043	P06469	Tropomyosin alpha chain	+11.5
SMk19	P02587	Troponin C	+14.5
SMk50	Y00760	Troponin-C	+19.6
SMk57	AAA91854	Troponin-C	+14.6
SM1535	P02554	Tubulin beta chain	+2.8
SM1063	P20152	Vimentin	-5.4
Metabolism			
SMk56	AAA37210	Aldolase A	+5.5
SM995	CAA59331	Carbonate dehydratase	+3.2
SMk344	NM_012839	Cytochrome C	+3.4
SM800	AAG53955	Cytochrome c oxidase subunit I	+3.0
SM51	T10974	Cytochrome-c oxidase	+3.8
SMk151	CAA06313	Fructose-1,6-bisphosphatase	+7.1
SM2070	P00339	L-lactate dehydrogenase M chain	+12.7
SMk120	AJ275968	LIM domains 1 protein	+8.6
SMk147	X59418	NADH dehydrogenase	+2.4
SM928	O79874	NADH-ubiquinone oxidoreductase chain 1	+5.3
SMk18	AAG28185	NADH4L	+2.1
SMk81	O19094	Octanoyltransferase(COT)	+3.2
SM295	AB006852	Phosphoarginine phosphatase	+2.6
SMk346	M97664	Phosphoglucomutase isoform 2 mRNA	+5.5
SM36	TVMVRR	Protein-tyrosine kinase	+4.3
SM887	P11980	Pyruvate kinase	+8.5
SM698	S64635	Pyruvate kinase	+9.7
SM723	P52480	Pyruvate kinase	+7.3
SMk79	U44751	Pyruvate kinase	+5.2
SMk135	Z98820	Sarcolipin	+3.0
SM1033	XM_018138	Tyrosine phosphatase type IVA	+2.9
SMk347	X99312	UDP glucose pyrophosphorylase	+3.0
Gene/protein expression			
SM75	U09823	Elongation factor 1 alpha	-4.3
SM1989	AAH05660	Elongation factor 1 alpha 1	-3.9
SMk61	NP_031959	Enolase 3	+3.6
SM968	Y00104	Repetitive dna sequence element RPE-1	-2.5

SMk91	AAC48501	Reticulum protein	+4.6
SM2083	NP_003083	Ribonucleoprotein polypeptide B	+3.1
SM896	AAH01127	Ribosomal protein	+2.0
SM1668	AAH07512	Ribosomal protein L18a	+2.1
SM1784	228176	Ribosomal protein P0	+6.2
SM1801	AAA30799	Transfer RNA-Trp synthetase	+6.0
SM997	51077272	Translation initiation factor eif1	+3.5
Cell signaling / communication			
SM464	AJ002189	Complete mitochondrial DNA	+3.9
SM732	AF304203	Mitochondrion	+5.9
SMk11	XM_006515	Potassium channel	-2.4
SMk187	BC007462	Similar to creatine kinase	+3.5
Cell division			
SM1067	XP_007399	Protease, cysteine, 1	+3.1
Immune response			
SM154	AF036005	Interleukin-2 receptor alpha chain	-2.5
SMk1	AAAG52886	Kel-like protein	+6.4
SM401	AJ251829	MHC class I SLA genomic region	-3.0
EST			
SM824	AK023385	cDNA FLJ13323 fis	+2.5
SM1776	XM_050494	KIAA0182 protein	+3.6
SM1556	XP_043678	KIAA1096 protein	+4.9
Unknown			
SM1785	AC015998	AC015998	+2.1
SM2152	BI327422	AR078G01iTHYEG01S	-4.0
SM1469	BG938561	Cn26h08.x1	-2.2
SM908	AAG28205	COI	+2.8
SM851	AAG28192	COI	+3.6
SM1738	CAA19420	DJ466P17.1.1(Laforin)	+4.8
SM1007	AAD31021	Foocen-m	+3.8
SM1920	BE421626	HWM012cA.1	+3.3
SM1972	XP_039195	Hypothetical protein	+3.2
SM1536	T08758	Hypothetical protein	+4.7
SMk137	XP_002275	Hypothetical protein	+20.0
SM1724	XP_016035	Hypothetical protein	-2.6
SM1539	AT001097	Mandarina library	-2.3
SM1474	BG384994	MARC 1PI	+2.6
SM1853	BF198401	MARC 2PIG	+3.6
SM1941	BE925069	MR1-AN0039-290800-004-a01	+4.4
SM379	AW328623	NIH_MGC_4	+2.3
SM1911	BE872239	NIH_MGC_65	-2.4
SM1676	BG548727	NIH_MGC_77	+5.1
SM1914	BG534187	NIH_MGC_77	-2.3
SM1650	BI337009	Peripheral Blood Cell cDNA library	+9.3
SM1064	BAB28119	Putative	+3.4
SM618	BAB28422	Putative	+2.1
SM1774	BAB30715	Putative	+3.2
SM1690	BF864360	Reinhardtii CC-1690	+2.2
SM1898	F23148	Small intestine cDNA library	-2.3
SM96	M17733	Thymosin beta-4 mRNA	-4.2
SM1922	AAH03026	Unknown	+4.0
SM210	BAA91923	Unnamed protein product	-3.1
No match			
SM107		No match	-2.4
SM278		No match	-2.2

SM384	No match	-2.3
SMk37	No match	+7.7
SM717	No match	-3.0
SM1598	No match	+4.5
SMk6	No match	+3.8
SMk68	No match	+5.0
SM1100	No match	-2.6
SMk70	No match	+3.9
SMk80	No match	+17.7
SMk112	No match	+3.5
SM1639	No match	-4.0
SMk148	No match	+3.8
SM1665	No match	+3.8
SM1665	No match	+13.0
SMk95	No match	+2.7
SMk133	No match	+2.4
SMk152	No match	+6.4
SM1897	No match	+3.4
SMk138	No match	+10.3
SM1902	No match	+2.1
SMk342	No match	+6.7
SMk181	No match	+11.0
SM904	No match	-3.4
SMk262	No match	+3.9
SM9	No match	+2.4
SM1964	No match	+2.6
SMk335	No match	-3.9

† : agreed Accession no.

**: Information agreed to the database

No match: No information agreed to the database; novel EST

ESM: early stage muscle (body weight 30 kg), ASM: adult stage muscle (body weight 90 kg), SM: swine muscle

【Table 2】

Expression ratio of differentially expressed genes between ESM and ESF

ESTs No.	Accession No†.	Description**	Ratio of gene expression ESF(30) / ESM(30)
Cellular structure and motility			
SM2149	CAB56598	1-alpha dynein heavy chain	-2.1
SM781	NP_033891	19 kDa-interacting protein 3-like	+2.2
SM1068	AAF20165	Actin	+4.5
SM635	BAB19361	Actin	+2.6
SM106	P53506	Actin	+4.9

SM768	X52815	Actin	+2.4
SM363	B25819	Actin	+3.7
SM713	AAA51586	Actin	+5.6
SMk77	NM_001100	Actin, alpha 1	+4.5
SM128	NP_033740	Actin, gamma 2	+3.9
SM1091	JC5971	Alpha-b crystallin	+2.1
SM902	BC001748	Annexin A2	-4.2
SM846	P81287	Annexin V	-3.5
SM653	P04272	Annexin II	-2.3
SMk340	U75316	Beta-myosin heavy chain mRNA	+2.2
SM1807	AAF99682	Calpain large polypeptide L2	+2.7
SM541	NP_000079	Collagen	-4.9
SM715	L47641	Collagen	-5.2
SM1023	Q9XSJ7	Collagen alpha 1	-4.6
SM758	CGHU1S	Collagen alpha 1	-4.3
SM62	CGHU2V	Collagen alpha 2	-4.4
SM949	O46392	Collagen alpha 2	-3.2
SM410	CAA28454	Collagen(alpha V)	-2.3
SM1121	NM_000393	Collagen, type V, alpha 2	-2.8
SM53	NP_000384	Collagen, type V, alpha 2	-2.5
SM1651	XM_039583	Discs, large(Drosophila) homolog 5	-8.6
SM1050	AAA30521	Fibronectin	-3.1
SM381	FNHU	Fibronectin precursor	-2.6
SM122	P07589	Fibronectin(FN)	-2.5
SM1573	XM_044160	Lamin A/C	+2.1
SMk55	NP_006462	Myosin	+3.6
SMk168	AB025261	Myosin heavy chain	+5.0
SM1732	NP_004678	Myotubularin related protein 4	+4.7
SM690	NP_003109	Secreted protein, acidic	-5.2
SM1043	P06469	Tropomyosin alpha chain	+8.6
SMk173	X66274	Tropomysin	+2.2
SMk19	P02587	Troponin C	+6.9
SMk57	AAA91854	Troponin-C	+7.1
SMk50	Y00760	Troponin-C	+9.0
SM1535	P02554	Tubulin beta chain	+3.3
SM1063	P20152	Vimentin	-5.1
SM730	CAA69019	Vimentin	-3.2
Metabolism			
SMk344	NM_012839	Cytochrome C	+2.4
SM800	AAG53955	Cytochrome c oxidase subunit I	+2.9
SMk151	CAA06313	Fructose-1,6-bisphosphatase	+4.2
SMk254	231300	Glycogen Phosphorylase b	+2.6
SM2070	P00339	L-lactate dehydrogenase M chain	+10.6
SM928	O79874	NADH-ubiquinone oxidoreductase chain 1	+3.2
SMk81	O19094	Octanoyltransferase(COT)	+3.9
SM295	AB006852	Phosphoarginine phosphatase	+2.3
SMk346	M97664	Phosphoglucomutase isoform 2 mRNA	+3.3
SM36	TVMVRR	Protein-tyrosine kinase	+2.6
SM723	P52480	Pyruvate kinase	+7.5
SM698	S64635	Pyruvate kinase	+6.6
SM887	P11980	Pyruvate kinase	+6.3
SM1594	AAA62278	Superoxide dismutase	-3.2
SM1033	XM_018138	Tyrosine phosphatase type IVA	+2.2
Gene/protein expression			
SM75	U09823	Elongation factor 1 alpha	-3.7

SM1989	AAH05660	Elongation factor 1 alpha 1	-3.8
SMk120	AJ275968	LIM domains 1 protein	+9.9
SMk91	AAC48501	Reticulum protein	+2.1
SM2083	NP_003083	Ribonucleoprotein polypeptide B	+3.2
SM21	NP_000994	Ribosomal	+2.2
SM1784	228176	Ribosomal protein P0	+5.5
SM1820	BC014277	Tissue inhibitor of metalloproteinase 3	-2.6
SM1801	AAA30799	Transfer RNA-Trp synthetase	+5.7
SM997	51077272	Translation initiation factor eif1	+2.3
Cell signaling / communication			
SM464	AJ002189	Complete mitochondrial DNA	+2.7
Immune response			
SMk1	AAG52886	Kel-like protein 23	+4.6
EST			
SM1776	XM_050494	KIAA0182	+3.2
SM1556	XP_043678	KIAA1096 protein	+4.5
Unknown			
SM2152	BI327422	AR078G01iTHYEG01S	-5.5
SMk3	AL13277	Chromosome 14 DNA sequence	+2.3
SM908	AAG28205	COI	+2.2
SM1738	CAA19420	DJ466P17.1.1(Laforin)	+3.5
SM1007	AAD31021	Foocen-m	+3.0
SM1724	XP_016035	Hypothetical protein	-2.6
SMk137	XP_002275	Hypothetical protein	+10.0
SM1972	XP_039195	Hypothetical protein	+2.8
SM787	AF192528	Integrin beta-1 subunit	+2.0
SM1474	BG384994	MARC 1PI	+2.8
SM1676	BG548727	NIH_MGC_77	+2.3
SM1650	BI337009	Peripheral Blood Cell cDNA library	+7.3
SM1774	BAB30715	Putative	+5.1
SM1064	BAB28119	Putative	+3.0
SM1690	BF864360	Reinhardtii CC-1690	+2.5
SM96	M17733	Thymosin beta-4 mRNA	-3.9
SM1922	AAH03026	Unknown	+4.7
No match			
SMk58		No match	+2.9
SM717		No match	-4.4
SMk6		No match	+2.4
SMk68		No match	+3.2
SMk80		No match	+4.3
SMk112		No match	+2.1
SM1639		No match	-2.8
SMk148		No match	+2.9
SM1665		No match	+9.8
SMk95		No match	+2.1
SMk152		No match	+6.4
SM1897		No match	+2.6
SMk138		No match	+3.1
SM796		No match	-2.2
SMk342		No match	+3.9
SMk181		No match	+4.4
SM904		No match	-2.7
SMk262		No match	+2.7
SM9		No match	+2.9
SM1964		No match	+2.6

SMk335	No match	+3.8

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**: Information agreed to the database

No match: No information agreed to the database; novel EST

ESM: early stage muscle (body weight 30 kg), ESF: early stage fat (body weight 30 kg), SM: swine muscle

From the above results, the present inventors shown the expression profiles of genes specifically expressed in the muscle and fat tissues of swine using the cDNA for screening and function analysis of swine genes according to the present invention and present the usability thereof in the improvement and evaluation of meat quality. Also, we identified the nucleotide sequences of growth-related factors and present the applicability thereof in the development of swine with excellent growth performance. In addition, by using the cDNA chip according to the present invention, it is expectedly possible to screen and compare expression profiles of genes according to swine breeds and tissues and to perform genetic mutation screening, genetic polymorphism interpretation, development of new drugs for disease treatment and disease diagnosis.

Example 2: Construction of a kit for screening and function analysis of swine genes

A kit for screening and function analysis of swine genes comprising the cDNA chip fabricated in Example 1, Cy5-dCTP or Cy3-dCTP bound cDNA from RNA of the tissue to be screened, a

fluorescence scanning system and a computer analysis system was fabricated.

Industrial Applicability

As explained through the Examples, the present invention relates to a cDNA chip for screening and function analysis of swine genes and provides a cDNA chip comprising a probe to detect marker genes specifically expressed in the muscle and fat tissues of swine, in which the probe is capable of complementarily bind to the marker genes. Also, the present invention provides expression profiles of marker genes which are related to economic traits of swine by using the cDNA chip according to the present invention. Therefore, the cDNA chip according to the present invention can be used for the comparison of genetic expression according to swine breeds and tissues, genetic mutation screening, genetic polymorphism interpretation, development of new drugs for disease treatment and disease diagnosis, swine improvement and thus, is very useful invention for the genetic engineering industry.